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USE OF INTERLEUKIN-11 TO TREAT GASTROINTESTINAL DISORDERS

RELATED APPLICATIONS

5 This application claims the benefit of the U.S. Provisional Application No. 60/129,421 filed April 15, 1999, the contents of which are incorporated herein by reference in their entirety.

FIELD OF INVENTION

10 The present invention relates to the field of prevention and treatment of gastrointestinal disorders using interleukin-11. More particularly, the present invention relates to preventing or treating gastrointestinal disorders using interleukin-11 to enhance motility of the digestive tract and/or contractility of the lower esophageal sphincter.

BACKGROUND OF THE INVENTION

Motilin, a gut polypeptide hormone, causes contraction of the stomach antrum and relaxation of the pyloric sphincter, thereby promoting gastric emptying. Toyota, K., *J. Smooth Muscle Res.* (1998) 34:13-22. Central nervous system input (afferent, efferent) is not necessary for cyclic interdigestive activity or cyclic release of motilin. Siadati, M. and M.G. Sarr, *J. Gastrointest. Surg.* (1998) 2:363-72. Motilin reduces fasting gall bladder volume and increases stomach antral contractions in humans. Luiking, Y.C., et al., *Gut* 20 (1998) 42:830-835. Motilin receptors are distributed throughout the rabbit brain, suggesting a neurotransmitter role for motilin in the brain. Depoortere, I., et al., *Brain Res.* (1997) 777:103-109.

In man, rabbit and cat, the effects of motilin and motilides are neurally mediated in vivo, whereas in vitro binding and contractility studies suggest the presence of a smooth muscular receptor. Motilin enhances contractions induced by electrical field stimulation 25 in the rabbit antrum by a post-ganglionic interaction with the cholinergic neurotransmission in vitro at low doses and interacts directly with antral smooth muscle at high doses. Van Assche, G., et al., *Eur. J. Pharmacol.* (1997) 337:267-274. Cholinergic and NANC inhibitory nerves play an important role in human lower esophageal sphincter

- 5 (LES) contraction, and motilin and cisapride may be clinically useful for improving the impaired LES of patients with gastroesophageal reflux. Tomita, R., et al., *Surg. Today* (1997) 27:985-992. Induction by motilin of phase III activity in human antrum is dependent on muscarinic mediation and the contractile effect of motilin on human duodenum involves a noncholinergic mechanism, as compared to the antral pathway.
- 10 Boivin, M., et al., *Am. J. Physiol.* (1997) 272:G71-6.

Cyclical motor activity of the gastrointestinal tract, normally occurring during the interdigestive period in several mammals, is disrupted in the post-operative ileus. After laparotomy, the cyclical motor activity recovers faster in the distal intestine than in the proximal intestine and the stomach, and that KW-5139 (a motilin derivative), but not

15 PGF₂-alpha (a naturally-occurring F-series prostaglandin) shortens the reappearance time of the phase III activity in the stomach. Yokoyama, T., et al., *Neurogastroenterol. Motil.* (1995) 7:199-210.

- Motilin is present in human breast milk at 100 pg/ml, and in the stomach its digestion is sufficiently retarded by human milk in the newborn to exert a biological role.
- 20 De Clercq, P., et al., *Life Sci.* (1998) 63:1993-2000. Minimal enteral feeding (MEF) favors secretion of gastrointestinal hormones in sick premature infants. Early MEF seems to be preferable to late one since it allows a faster secretion related to volume of the formula. Ordaz-Jimenez, M.R., et al., *Rev. Invest. Clin.* (1998) 50:37-42. Although the motilin receptor appears to be functionally present beyond 32 weeks of gestation, as assessed by
- 25 in indirect pharmacologic challenge, hormonal modulation of migrating activity in the neonate by plasma motilin and pancreatic polypeptide is absent. Jadcherla, S.R., et al., *Pediatr. Res.* (1997) 42:365-9.

The exact pathophysiology of motility disorders, such as those described above, is not well understood. Consequently, a rational therapy for treating these disorders is also

30 not available. Pharmacological agents which enhance the motility in the paralytic gut may be useful in the treatment and prevention of gastrointestinal disorders such as gastroesophageal reflux disease and surgery-induced adynamic ileus (also known as post-operative period ileus). Motility-enhancing agents (also known as gastroprokinetic agents) may also be useful in preventing or treating feeding intolerance in preterm infants.

5 One common approach to treating gastroesophageal reflux disease involves the use of the antiemetic agent metoclopramide, a benzamide having dopamine D2-receptor antagonist activity. Unfortunately, metoclopramide has several side effects, including an increase in prolactin levels and development of dyskinesia.

10 Another common practice for treating motility disorders involves the use of macrolide antibiotics, such as erythromycin. However, macrolide antibiotics are known to cause abdominal cramps and diarrhea, thus limiting their clinical application. Whether these side effects are secondary to their antibiotic activity or are due to their effect on gastrointestinal motility and secretion is not known. Various attempts to produce erythromycin derivatives having improved gastropromkinetic properties have met with
15 limited success. See, e.g., Omura et al., *J. Med. Chem.* (1987) 30(11):1941-1943; and Omura et al., *J. Antibiotics* (1987) 38(11):1631-1632.

 Thus, there remains a need for an effective, clinically applicable means of preventing or treating gastrointestinal disorders characterized by a defective motility pattern.

20

SUMMARY OF THE INVENTION

 Applicants have for the first time determined that interleukin-11 ("IL-11") increases plasma levels of motilin, a known gastrointestinal prokinetic gut hormone. Thus, IL-11 will enhance motility in the paralytic gut and increase contractility of gastrointestinal muscles, such as the lower esophageal sphincter and stomach antrum. As a result, IL-11
25 can be used to treat diseases and other conditions which result from defective motility patterns, such as, for example, in treating or preventing gastroesophageal reflux disease, post-operative adynamic ileus, and feeding intolerance in preterm infants.

 Provided by the present invention are methods of treating disorders where an increase in plasma level of motilin is shown to be beneficial including, without limitation,
30 gastroesophageal reflux disease, post-operative adynamic ileus, and feeding intolerance in preterm infants.

 According to the present invention, IL-11, analogs, and derivatives thereof, are administered to patients, either prophylactically or at the onset of symptoms associated with the aforementioned disorders. IL-11 can be administered in suitable pharmaceutically

- 5 acceptable carriers either alone or in combination with other conventional agents useful in alleviating the symptoms associated with the aforementioned disorders.

In one embodiment, the invention comprises a method of preventing a gastrointestinal disorder which comprises administering to a mammal, prior to the on-set of symptoms, a therapeutically effective amount of interleukin-11.

- 10 In another embodiment, the invention comprises a method of treating a gastrointestinal disorder which comprises administering to a mammal experiencing a gastrointestinal disorder a therapeutically effective amount of interleukin-11.

In preferred embodiments, the therapeutic dose is effective to prevent or treat a gastrointestinal disorder resulting from defective gastrointestinal motility or reduced
15 contractility of the lower esophageal sphincter or duodenum. Preferably, the therapeutically effective amount of interleukin-11 comprises between about 1 and 1000 $\mu\text{g/kg}$ body weight, and more preferably between about 1 and 100 $\mu\text{g/kg}$ body weight.

DETAILED DESCRIPTION OF THE INVENTION

The following abbreviations are used herein: interleukin-11 (IL-11); recombinant
20 human IL-11 (rhIL-11); interleukin-12 (IL-12); tumor necrosis factor (TNF); interferon (IFN); trinitrobenzene sulfonic acid (TNBS); substance P (SP); acetylcholine (ACh); non-adrenergic non-cholinergic (NANC); lower esophageal sphincter (LES); and prostaglandin (PG).

All patent and literature references cited are incorporated herein by reference as if
25 fully set forth.

Provided by the present invention are methods of treating disorders where an increase in plasma level of motilin is shown to be beneficial including, without limitation, gastroesophageal reflux disease, post-operative adynamic ileus, and feeding intolerance in preterm infants.

30 IL-11 is a stromal cell-derived pleiotropic cytokine which interacts with a variety of hematopoietic and non-hematopoietic cell types. Recombinant human IL-11 stimulates megakaryocytopoiesis *in vitro* and *in vivo*. Weich, N. S., et al. (1997) *Blood* 90:3893-3902; and Orazi, A., et al. (1996) *Exp. Hematol.* 24:1289-1297. IL-11 also stimulates erythropoiesis and regulates macrophage proliferation and differentiation. de Haan, G.,

- 5 et al. (1995) *Br. J. Haematol.* 90:783-790. Due to its thrombopoietic activities *in vivo*, IL-11 is used to treat chemotherapy-induced thrombocytopenia. Kaye, J. A. (1996) *Curr. Opin. Hematol.* 3:209-215.

In addition to its hematopoietic effects, IL-11 also protects against various forms of mucosal epithelial cell injury. For example, IL-11 has been shown to protect small
 10 intestinal cells from combined radiation, chemotherapy, and ischemia (Du, X., et al. (1997) *Am. J. Physiol.* 272:G545-G552; Orazi, A., et al. (1996) *Lab. Invest.* 75:33-42; and Keith, J. C., Jr., et al. (1994) *Stem. Cells. (Dayt)*. 1(12):79-89); reduce experimental colitis induced by trinitrobenzene sulfonic acid in rat (Qiu, B. S., et al. (1996) *Dig. Dis. Sci.* 41:1625-1630); and ameliorate inflammatory bowel disease (Orazi, A., et al. (1996) *Lab.*
 15 *Invest.* 75:33-42). The foregoing studies show that treatment with IL-11 decreases mucosal damage, accelerates healing and improves host survival. IL-11 also reduces immune-mediated small bowel injury in acute GVHD following murine allogeneic bone marrow transplantation. Hill, G. R., et al. (1998) *J. Clin. Invest.* 102:115-123.

IL-11 has also been shown to improve survival and decrease TNF production after
 20 radiation-induced thoracic injury. Redlich, C. A., et al. (1996) *J. Immunol.* 157:1705-1710. Human IL-11, expressed as a transgene in bronchial mucosa, reduces mortality associated with hyperoxia in mice. Waxman, A. B., et al. (1998) *J. Clin. Invest.* 101:1970-1982. This enhanced murine survival may result from reduced lung injury, including alveolar-capillary protein leak, endothelial and epithelial cell membrane injury, lipid
 25 peroxidation, pulmonary neutrophil recruitment, IL-12 and TNF production, and DNA fragmentation.

The mechanisms by which IL-11 protects mucosal membranes are not fully understood. IL-11's anti-inflammatory effects are believed to result, at least in part, from down-regulation of various proinflammatory cytokines. Leng, S. X. and J. A. Elias (1997)
 30 *J. Immunol.* 159:2161-2168; Trepicchio, W. L., et al. (1997) *J. Immunol.* 159:5661-5670; and Trepicchio, W. L., et al. (1996) *J. Immunol.* 157:3627-3634. IL-11 may also cause immune deviation from a T_H1 -like to a T_H2 -like phenotype, thereby alleviating immune-mediated injury. Hill, *supra*.

IL-11 belongs to the interleukin-6 (IL-6) family of cytokines, all of which use
 35 gp130 as a critical component for signal transduction. Taga, T. and T. Kishimoto (1997)

5 *Annu. Rev. Immunol.* 15:797-819; Zhang, X. G., et al. (1994) *J. Exp. Med.* 179:1337-1342;
 and Yang, Y. C. and T. Yin (1995) *Ann. N.Y. Acad. Sci.* 762:31-40. IL-11 initiates
 signaling via binding to a unique IL-11-receptor- α (IL-11R α) chain. Nandurkar, H. H.,
 et al. (1996) *Oncogene* 12:585-593; Miyatake, T., et al. (1998) *J. Immunol.* 160:4114-
 4123. The IL-11/IL-11R α complex is thought to bind to and induce clustering gp130,
 10 leading to the activation, via transphosphorylation, of associated JAKs. Yin, T., K., et al.
 (1994) *Exp. Hematol.* 22:467-472; Wang, X. Y., et al. (1995) *J. Biol. Chem.* 270:27999-
 28002. Activated JAKs phosphorylate tyrosine residues within the cytoplasmic region of
 gp130 which then serve as docking sites for signal transducer and activators of
 transcription proteins, STAT3 and STAT1. Luticken, C., et al. (1994) *Science* 263:89-92;
 15 Hemmann, U., et al. (1996) *J. Biol. Chem.* 271:12999-13007. The activated JAKs
 subsequently phosphorylate tyrosine residues within the bound STAT proteins, causing the
 STATs to dissociate from gp130, dimerize, and enter the nucleus to act as transcriptional
 activators of target genes. Zhong, Z., et al. (1994) *Science* 264:95-98; Ihle, J.N. (1996)
Cell 84:331-334; and Akira, S. (1997) *Int. J. Biochem. Cell Biol.* 29:1401-1418. STAT
 20 dimers may be additionally phosphorylated on serine or threonine residues by mitogen
 activated protein kinases (MAPKs) that are also activated in response to cytokine binding
 to the receptor. Zhang, X., et al. (1995) *Science* 267:1990-1994; Boulton, T. G., et al.
 (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92:6915-6919; Adunyah, S. E., et al. (1995) *Ann. N.Y.*
Acad. Sci. 766:296-299; and Yin, T. and Y. C. Yang (1994) *J. Biol. Chem.* 269:3731-3738.
 25 This additional phosphorylation may potentiate STAT function as an activator of
 transcription.

IL-11 is described in detail in International Application PCT/US90/06803,
 published May 30, 1991; as well as in U.S. Patent No. 5,215,895; issued June 1, 1993. A
 cloned human IL-11 was previously deposited with the ATCC, 10801 University
 30 Boulevard, Manassa, VA 20110-2209, on March 30, 1990 under ATCC No. 68284.
 Moreover, as described in U.S. Patent No. 5,270,181; issued December 14, 1993; and U.S.
 Patent No. 5,292,646; issued March 8, 1994; IL-11 may also be produced recombinantly
 as a fusion protein with another protein. IL-11 can be produced in a variety of host cells
 by resort to now conventional genetic engineering techniques. In addition, IL-11 can be
 35 obtained from various cell lines, for example, the human lung fibroblast cell line, MRC-5

5 (ATCC Accession No. CCL 171) and Paul *et al.*, the human trophoblastic cell line, TPA30-1 (ATCC Accession No. CRL 1583). Described in Proc Natl Acad Sci USA 87:7512 (1990) is a cDNA encoding human IL-11 as well as the deduced amino acid sequence (amino acids 1 to 199). U.S. Patent No. 5,292,646, *supra*, describes a des-Pro form of IL-11 in which the N-terminal proline of the mature form of IL-11 (amino acids
10 22-199) has been removed (amino acids 23-199). As is appreciated by one skilled in the art, any form of IL-11, which retains IL-11 activity, is useful according to the present invention.

In addition to recombinant techniques, IL-11 may also be produced by known conventional chemical synthesis. Methods for constructing the polypeptides useful in the
15 present invention by synthetic means are known to those of skill in the art. The synthetically constructed cytokine polypeptide sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with the natural cytokine polypeptides are anticipated to possess biological activities in common therewith. Such synthetically constructed cytokine polypeptide sequences or fragments thereof, which
20 duplicate or partially duplicate the functionality thereof may also be used in the method of this invention. Thus, they may be employed as biologically active or immunological substitutes for the natural, purified cytokines useful in the present invention.

Modifications in the protein, peptide or DNA sequences of these cytokines or active fragments thereof may also produce proteins which may be employed in the
25 methods of this invention. Such modified cytokines can be made by one skilled in the art using known techniques. Modifications of interest in the cytokine sequences, *e.g.*, the IL-11 sequence, may include the replacement, insertion or deletion of one or more selected amino acid residues in the coding sequences. Mutagenic techniques for such replacement, insertion or deletion are well known to one skilled in the art. (See, *e.g.*, U. S. Patent No.
30 4,518,584.)

Other specific mutations of the sequences of the cytokine polypeptides which may be useful therapeutically as described herein may involve, *e.g.*, the insertion of one or more glycosylation sites. An asparagine-linked glycosylation recognition site can be inserted into the sequence by the deletion, substitution or addition of amino acids into the peptide
35 sequence or nucleotides into the DNA sequence. Such changes may be made at any site

5 of the molecule that is modified by addition of O-linked carbohydrate. Expression of such altered nucleotide or peptide sequences produces variants which may be glycosylated at those sites.

Additional analogs and derivatives of the sequence of the selected cytokine which would be expected to retain or prolong its activity in whole or in part, and which are
10 expected to be useful in the present method, may also be easily made by one of skill in the art. One such modification may be the attachment of polyethylene glycol (PEG) onto existing lysine residues in the cytokine sequence or the insertion of one or more lysine residues or other amino acid residues that can react with PEG or PEG derivatives into the sequence by conventional techniques to enable the attachment of PEG moieties.

15 Additional analogs of these selected cytokines may also be characterized by allelic variations in the DNA sequences encoding them, or induced variations in the DNA sequences encoding them. It is anticipated that all analogs disclosed in the above-referenced publications, including those characterized by DNA sequences capable of hybridizing to the disclosed cytokine sequences under stringent hybridization conditions
20 or non-stringent conditions (Sambrook *et al.*, Molecular Cloning. A Laboratory Manual, 2d edit., Cold Spring Harbor Laboratory, New York (1989)) will be similarly useful in this invention.

Also considered useful in these methods are fusion molecules, prepared by fusing the sequence or a biologically active fragment of the sequence of one cytokine to another
25 cytokine or proteinaceous therapeutic agent, *e.g.*, IL-11 fused to IL-6 (see, *e.g.*, methods for fusion described in PCT/US91/06186 (WO92/04455), published March 19, 1992). Alternatively, combinations of the cytokines may be administered together according to the method.

Thus, where in the description of the methods of this invention IL-11 is mentioned
30 by name, it is understood by those of skill in the art that IL-11 encompasses the protein produced by the sequences presently disclosed in the art, as well as proteins characterized by the modifications described above yet which retain substantially similar activity.

Pharmaceutical compositions containing IL-11 which are useful in practicing the methods of the present invention may also contain pharmaceutically acceptable carriers,
35 diluents, fillers, salts, buffers, stabilizers and/or other materials well-known in the art. The

5 term "pharmaceutically acceptable" means a material that does not interfere with the effectiveness of the biological activity of the active ingredient(s) and that is not toxic to the host to which it is administered. The characteristics of the carrier or other material will depend on the route of administration.

It is currently contemplated that the various pharmaceutical compositions should
10 contain about 0.1 micrograms to about 1 milligram per milliliter of the active ingredient.

Administration can be carried out in a variety of conventional ways. Intraperitoneal injection is the preferred method of administration. Intravenous, cutaneous or sub-cutaneous injection may also be employed. For injection, IL-11 will preferably be administered in the form of pyrogen-free, parenterally acceptable aqueous solutions. The
15 preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

The amount of IL-11 used for treatment will depend upon the severity of the condition, the route of administration, the reactivity or activity of the active ingredient, and ultimately will be decided by the treatment provider. In practicing the methods of
20 treatment of this invention, a therapeutically effective amount of IL-11 is administered. The term "therapeutically effective amount" means the total amount of each active component of the method or composition that is sufficient to show a meaningful patient benefit (e.g., curing, ameliorating, inhibiting, delaying or preventing onset of, preventing recurrence or relapse of). One common technique to determine a therapeutically effective amount for
25 a given patient is to administer escalating doses periodically until a meaningful patient benefit is observed by the treatment provider. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.
30 A therapeutically effective dose of IL-11 in this invention is contemplated to be in the range of about 1 to about 1000 $\mu\text{g/kg}$ body weight, and more preferably between about 1 and about 100 $\mu\text{g/kg}$ body weight. The number of administrations may vary, depending on the individual patient and the severity of the gastrointestinal disorder.

The present invention is further exemplified and supported by reference to the
35 experimental results described below.

EXAMPLES

Example 1: Effect of IL-11 on TNBS-induced colitis in rabbits

IL-11 is believed to attenuate the inflammatory response via a reduction of the release of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-12 and IFN- γ) and of nitric oxide production by macrophages. Trepicchio, W.L., et al., *J. Immunol.* (1997) 157:3627-
10 3634;
J. Immunol. (1997) 159:5661-5670. This example demonstrates that IL-11 affects inflammatory changes in the deeper, neuromuscular layers of the gut wall. Specifically, this example shows the effects of treatment with IL-11 in New Zealand rabbits with colitis induced by intrarectal application of TNBS. Muscle strips from the inflamed region have
15 an increased passive tension, and a decreased contractile response to ACh, motilin, SP and potassium ion. Subcutaneous infusion of 40 μ g/kg per day IL-11 (or more), for 5 days following induction of inflammation, normalizes the contractile parameters. The response towards motilin and SP was normalized with a dose of 40 μ g/kg per day; the response to ACh and potassium ion was normalized with a dose of 720 μ g/kg per day. The decrease
20 in motilin and SP receptor density was also reversed by IL-11 treatment. Treatment with IL-11 dose-dependently decreased weight in these rabbits. Depoortere, I., et al., *Am. Gastroenterology Soc.* (New Orleans, LA, May 16-22, 1998).

Example 2: Effect of IL-11 on plasma and tissue concentrations of motilin and SP

The effects of IL-11 treatment on plasma and tissue concentrations of motilin and
25 SP present in endocrine cells and/or neurons of the gut wall were investigated. Depoortere, I., et al., *Am. Gastroenterology Soc.* (to be presented May 15-20, 1999, Orlando, FL). Rabbits received 4, 40, 72 or 720 μ g/kg recombinant IL-11 sc. or saline (control). One hour later, colitis was induced with 135 mg/kg TNBS and a sc. infusion of 4, 40, 72 or 720 μ g/kg per day IL-11 or saline was started for 5 days. SP and motilin were measured by
30 RIA, before the induction of inflammation and just before the rabbits were sacrificed, in plasma and in extracts prepared from the mucosa of the duodenum and the colon and from the muscle layer of the colon. mRNA levels were determined by semi-quantitative RT-PCR. IL-11 concentrations were measured by ELISA. Plasma motilin levels were not

5 influenced by the inflammatory process (649 ± 69 vs 724 ± 126 pg/ml). The motilin content was increased from 381 ± 78 to 664 ± 74 ng/g tissue in the duodenal mucosa, but not in the mucosa (64 ± 4 vs 78 ± 12 ng/g tissue) or muscle layer (24 ± 4 vs 17 ± 1 ng/g tissue) of the inflamed colon. Inflammation also increased motilin mRNA expression 2.5 fold in the duodenal mucosa. In contrast, plasma SP levels were decreased from 1812 ± 60 to
10 635 ± 101 pg/ml, SP content in the muscle layer of the colon from 45 ± 8 to 7 ± 2 ng/g tissue. In the duodenal or colonic mucosa SP content was unchanged. Treatment of rabbits during colitis with IL-11 (4, 40, 72, 720 μ g/kg per day) resulted at day 5 in an increase in plasma IL-11 levels of respectively 218 ± 91 , 5345 ± 1876 , 10221 ± 2175 , 116527 ± 25461 pg/ml and increased plasma motilin levels with 199 ± 77 , 799 ± 201 , 1740 ± 560 and 2084 ± 797 pg/ml.
15 IL-11 treatment also dose-dependently augmented the motilin content in the duodenal mucosa from 664 ± 74 (TNBS) to 783 ± 65 , 1070 ± 60 , 1176 ± 148 and 1273 ± 50 ng/g tissue. Similar observations were made in the colonic mucosa but not in the colonic muscle layer. This increase was not reflected in a further increase in motilin mRNA expression. However, a stimulatory effect of IL-11 was not observed on plasma SP levels which were
20 still decreased to 848 pg/ml with the highest dose of IL-11 tested, nor on the SP content in the duodenal or colonic mucosa. Only in the colonic muscle layer a small but significant increase was observed with low doses of IL-11. IL-11 treatment during colitis markedly increased plasma motilin levels and the motilin content in the mucosa of the duodenum and the colon. However, this effect was not observed with SP suggesting that
25 it is due to a specific interaction of IL-11 with the motilin endocrine cell which does not occur at the level of the motilin mRNA expression.

While the present invention has been described in terms of specific methods and compositions, it is understood that variations and modifications will occur to those skilled in the art upon consideration of the present invention. Numerous modifications and
30 variations in the invention as described in the above illustrative examples are expected to occur to those skilled in the art and, consequently, only such limitations as appear in the appended claims should be placed thereon. Accordingly, it is intended in the appended claims to cover all such equivalent variations which come within the scope of the invention as claimed.